nor antivirals are available to date, controlling norovirus infection presents significant challenges to public health. Norovirus protease (NVpro) plays a central role in the virus infectious cycle through a proteolytic maturation of nonstructural proteins that are not available in host cells. Therefore NVpro has been highlighted as a potential target for the antiviral drug development. While X-ray studies of the NVpro give us a wealth of the structural information including several key interactions for the substrate binding, the relevance of protein dynamics in the substrate biding and the ability to interact with multiple substrates has remained to be cleared. We present here the NMR solution structure and backbone dynamics of the protease from Norwalk virus, a prototype strain (GI.1) of norovirus. The bII-cII loop in C-terminal domain containing S2 binding site shows a nontrivial structural variation when compared to crystal structures and among lower energy structure ensemble. The possible biological implication of the observed dynamic properties of NVpro for the substrate recognition will be discussed.

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Decoding the Components of Dynamics in Three-Domain Proteins

Mateusz Maciejewski[1,2], Nico Tjandra[1], Paul N. Barlow[2].
[1]National Institutes of Health, Bethesda, MD, USA,
[2]University of Edinburgh, Edinburgh, United Kingdom.

One critical determinant of protein function is dynamics. In numerous cases, the binding of ligands (or drug molecules) to their partner proteins is accompanied by not only a change in the dynamics of sites near their relevant binding pockets, but also often by major conformational changes that readjust the shape of the binding pocket to better fit the ligand. Indeed, it is possible to break down the motion of proteins into several different categories of motion, including overall protein tumbling, relative motion of individual domains within the protein, concerted motion of loops and secondary structure elements within each domain, and finally internal motion of each single residue. Numerous studies (predominantly using NMR methods such as residual dipolar couplings and paramagnetic relaxation enhancement) have shown that proteins in their unbound states can also transiently explore their bound conformations via such intrinsic dynamics, a phenomenon not observable from the averaged structures produced in typical structural studies. It is therefore obvious that the exploration of all categories of protein motion is a very important step in the characterization of a given protein, as well as in rational drug design. There have been several efforts to theoretically describe and simulate such motion, mostly in the case of a single domain, and in one case two domains. In this study, we have examined the components of motion in more complex systems, by simulating the motion of three domains connected by various types of potentials and analyzing their correlation functions with extended model free formalism. This analysis method allows us to decompose motion in terms of physically meaningful parameters without making assumptions based on any particular models of motion.

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A Molecular View of Glycosylation and Glycation Effects on the Structure of Human Acetylcholinesterase

Cesar Millan-Pacheco, Benjamin Perez-Aguilar,
Guillermina Palomec-Sánchez, Noe Salinas-Arreortua,
Rosa María López-Durán, José Luis Gomez Olivares.
Universidad Autónoma Metropolitana, Mexico, DF, Mexico.

All the organisms react to distinct signals from the environment. Fast reactions to those signals dictate the difference between life and death. Signaling cascade is modulated, among other molecules, by proteins. One of the most efficient enzymes is the acetylcholinesterase (ache) that catalyzes the hydrolysis of acetylcholine to choline and acetate. It is found in multiple molecular forms that determinate its function and location over the body. Ache needs to be modified postraductionally (glycosylation) to be active; being refers to the enzymatic process that binds monosaccharides on asparagines residues of the protein structure (n-glycosilation). Aditionally, glucose may be incorporated on lysine residues to protein by an inespecific process (glycation).

In this work, we present the results of molecular simulations of ache in solution with different levels of glycosylation and glycation. Glycosylation was probed on 3 positions and glycation on 3 structurally conserved positions and all lysine residues solvent exposed of ache. 100ns of molecular simulations were done with explicit solvent at 298 k and 1 atm. The effect of sugar binding on the side chain residues of the binding site is presented.

Cesar, MP. acknowledges to conacyt and Uam-I for Postdoctoral funding and Aitzaloa and National Computer Centre (IPICYT) for computer time and to Eduardo Jardon Valadez for his help on the early stages of this work.

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Dynamics of Homologous and Heterologous Calcium Channel α_1 and β Subunits in the Skeletal Muscle Triad

Marta Campiglio[1], Valentina Di Biase[1], Petronel Tuluc[2],
Bernhard E. Flucher[1].
[1]Department of Physiology and Medical Physics, Medical Innsbruck University, Innsbruck, Austria, [2]Department of Pharmalogy and Toxicology, Innsbruck University, Innsbruck, Austria.

Skeletal muscle EC-coupling requires direct interactions of the dihydropyridine receptor (DHPR) with the ryanodine receptor (RyR1) and its organization in tetrads opposite RyR1. Accordingly, tetrad formation and skeletal muscle EC-coupling fail in myotubes expressing the cardiac α_{1C} subunit or lacking the β_{1a} subunit. This indicates that tissue-specific protein-protein interactions between α_{1S}, β_{1a} and RyR1 are important for the structure and function of the skeletal muscle EC-coupling apparatus. Here we apply fluorescence recovery after photobleaching (FRAP) in dysgenic ($\alpha_{1S}^{-/-}$) myotubes reconstituted with GFP-tagged DHPR subunits to probe the importance of these interactions in stabilizing them in the triad. Consistent with its anchoring in the triad, the α_{1S} subunit is essentially immobile. Surprisingly, also the cardiac α_{1C} subunit, which does not interact with RyR1, is immobile. Thus, the II-III loop - RyR1 interaction is not the determing factor for stabilizing α_{1S} in the triad. When coexpressed with α_{1S}, the β_{1a} subunit showed a higher fluorescence recovery rate than the α_1 subunits, suggesting that it can be dynamically exchanged in the complex. Again, the recovery did not increase when β_{1a} was coexpressed with α_{1C}, indicating that its putative interaction with RyR1 does not contribute to the stability of β_{1a} in the complex. Previously we showed that all βisoforms can associate with α_{1S} and α_{1C} in the triads. When the non-muscle β_{2a} or β_{4b} subunits were expressed with α_{1S} in dysgenic myotubes their recovery after photobleaching was significantly higher than that of β_{1a}, demonstrating that the homologous β subunit binds the channel complex with higher affinity than heterologous β subunits. Funding: FWF P20059, W1101, T443-B18.

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Analysing Helix Flexibility in Membrane Proteins using Bendix

A. Caroline E. Dahl, Matthieu Chavent, Mark S.P. Sansom.
University of Oxford, Oxford, United Kingdom.

Alpha helix flexibility plays a key role in the mechanisms of gating of ion channels and solute transporters. Examples of this can be seen in the voltage-gated potassium channel Kv1.2 and the mechanosensitive channel of small conductance, MscS, where molecular hinges within channel-lining helices are important for gating. Kv1.2 and MscS are not exceptional - membrane proteins often feature curved or kinked helices, and dynamics of such âœnon-idealâ helices is critical to protein function. However, non-linear helices are compromised by current visualization techniques, and call for better characterization. Bendix is a software that colours, quantifies and simplifies both static structures and dynamic (MD) simulation trajectories, without sacrificing details of local conformation. It also enables visualization of secondary structure in coarse-grained simulations of proteins. It is available as a plugin for the molecular graphics program Visual Molecular Dynamics (VMD, http://www.ks.uiuc.edu/Research/vmd) and works under most operating systems. Bendix offers custom colour schemes that highlight relevant helix curvature and allow you to explore protein structures. Generated helix data is easily presented using the in-built 2D or 3D graphing module, alternatively exported to other graphics packages. We will illustrate its application to a number of channel and transport proteins. including MscS, the potassium channel KcsA, the MFS transporter proteins L-fucose permease (FucP) and lactose permease (LacY), and the sodium-hydantoin transporter Mhp1. Bendix is user-friendly, free and available for download at http://sbcb.bioch.ox.ac.uk/dahl.php.

Protein Folding & Stability I

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Low Folding Cooperativity of HP35 Revealed by Single Molecule Force Spectroscopy and Molecular Dynamics Simulation

Chunmei Lv, Cheng Tan, Meng Qin, **Yi Cao**, Wei Wang.
Nanjing University, Nanjing, China.

Some small proteins, such as HP35, fold at submicrosecond time scale with low folding cooperativity. Although these proteins have been extensively investigated, still relatively little is known about their folding mechanism. Here using single molecule force spectroscopy and steered molecule dynamics simulation, we study the unfolding of HP35 under external force. Our results show that